GENETIC AND CLINICAL VARIATIONS IN A NORWEGIAN SAMPLE DIAGNOSED WITH RETT SYNDROME

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ABSTRACT

Background and purpose: Rett syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in *MECP2*. The diagnostic criteria of RTT are clinical; mutations in *MECP2* are neither diagnostic nor necessary, and a mutation in another gene does not exclude RTT. We attempted to correlate genotype and phenotype to see if there are significant clinical differences.

Methods: All available females diagnosed with RTT in Norway were invited to the study. Parents were interviewed, the girl or woman with RTT examined and medical records reviewed. All diagnoses were revisited according to the current diagnostic criteria and exome-based sequencing analyses were performed in individuals without an identified causative mutation. Participants were categorized according to genotypes and RTT diagnosis. Individuals with RTT with and without mutations in *MECP2* were compared.

Results: 91 individuals were included. A presumed causative mutation was identified in 86 individuals, of these, mutations in *MECP2* in 77 individuals and mutations in *SMC1A, SYNGAP1, SCN1A, CDKL5, FOXG1* or chromosome 13q in nine. Seventy-two individuals fulfilled the diagnostic criteria for classic and 12 for atypical RTT. Significant differences in early development, loss of hand use and language, intense eye gaze and the presence of early onset epilepsy were revealed in individuals with RTT according to their MECP2 genotypic status.

Conclusion: Using the current diagnostic criteria, genetic and clinical variation in RTT is considerable. Significant differences between individuals with RTT with and without *MECP2* mutations indicate that *MECP2* is a major determinant for the clinical phenotype in individuals with RTT.

HIGHLIGHTS

- Clinical features differ significantly in RTT with and without *MECP2* mutations
- Epilepsy has later onset in individuals with RTT with *MECP2* mutations
- Deviant early development is less common in individuals with RTT with *MECP2* mutations
- Six individuals with RTT had mutations in *SMC1A, SYNGAP1, SCN1A, CDKL5* or *FOXG1*

KEYWORDS

Rett syndrome, MECP2, Genetic variation, Clinical phenotype, Exome sequencing, Epilepsy

1. INTRODUCTION

For many years the neurodevelopmental disorder Rett syndrome (RTT, OMIM 312750) has been known as a clinical entity mainly caused by mutations in the *MECP2* gene [1]. The disorder almost exclusively affects females, and in its classic form, it is characterized by apparently normal development in the first 6-18 months of life before a regression occurs and acquired skills disappear [2].

The phenotypic spectrum of RTT has evolved since the first description of 22 girls with a homogenous phenotype by Andreas Rett in 1966 [3]. As the number of individuals diagnosed with RTT increased, the phenotype widened, and in 1994 the diagnosis included both classic and atypical RTT [4]. The current diagnostic criteria were published in 2010 [2]. In the last decade, the term RTT-like disorders have been used for individuals sharing many clinical characteristics with RTT, but not fulfilling the diagnostic criteria. In contrast to classic and atypical RTT, the term RTT-like disorder is not clearly defined [5].

Also the genotypic spectrum has extended in RTT. In 2004 and 2008, strong associations were found between atypical RTT and mutations in *CDKL5* and *FOXG1*, respectively [6, 7]. In the last decade, Next Generation Sequencing (NGS) has contributed to the identification of mutations in more than 100 genes other than *MECP2, CDKL5* and *FOXG1* in individuals with RTT or a RTT-like phenotype. Almost half of these as the only identified pathological mutation in individuals fulfilling the diagnostic criteria of classic or atypical RTT [5, 8-16]. The strong association between *MECP2* and RTT is however undisputable, with mutations in *MECP2* found in more than 95% of individuals with classic and 70-90% of individuals with atypical RTT [2].

A large number of studies have addressed the genotype in *MECP2* negative individuals within the RTT spectrum. There are, however, fewer studies comparing the phenotypes of these individuals to the phenotypes of individuals with *MECP2* mutations. Differences in phenotype between individuals with RTT with and without *MECP2* mutations have been reported, especially in early development and in epilepsy [17, 18]. In addition, differences between individuals with and without *MECP2* mutations have been explored in cohorts not based on RTT phenotypes [19]. With the increased number of genes associated with RTT and the increased number of individuals without RTT with a mutation in *MECP2*, more knowledge on phenotype-genotype correlations on the genetic level is important for the accuracy in diagnostics.

The present study investigates a population of females diagnosed with RTT through the last three decades. It examines all participants for the phenotypic traits contained in the 2010 diagnostic criteria for RTT, revisits their diagnoses and performs genomic investigations in individuals without an identified causative mutation. In addition, it compares the phenotypes of

individuals with and without a *MECP2* mutation in the entire RTT group as well as within the RTT diagnostic subgroups of classic and atypical RTT.

2. METHODS

2.1 Participants

Recruitment took place from 2013 to 2017. Invitation to participate was distributed to families or guardians of females with RTT or a RTT-like disorder through the Norwegian Rett Syndrome Association (n=126) and Frambu, the Norwegian Resource Centre for Rare Disorders (n=116). The rate of overlapping between the two search groups was high, as only 165 subjects with RTT had been reported to the Norwegian Patient Registry from the Specialist Health Services in 2013. Lists of names from these sources were not revealed to the study group. In addition, some families with a member with RTT were referred from habilitation clinics and neurologists and some families contacted the authors directly. Review of the diagnosis was based on the latest consensus criteria [2]. Individuals sharing some clinical features with RTT, but not fulfilling the diagnostic criteria were described as non-RTT.

2.2 Procedures

Parents/caregivers were first asked to complete a questionnaire. A meeting with the family at the local hospital or in the home was arranged where a clinical examination was performed together with a semi-structured interview with parents/caregivers. A review of the participants' medical records was carried out to complete the data sets.

2.3 Measures

The clinical examination included growth parameters, level of contact, presence of stereotypies and respiration abnormalities, as well as assessment of muscle tone, deep tendon reflexes, coordination and scoliosis. The interview addressed pregnancy and birth, development of communication and language skills, clinical symptoms and results of previous genetic workup, to the best knowledge of the family. The questionnaire comprised information about demographic background and development of motor skills. Head circumference was categorized using normative z-scores [20]. Disease severity was quantified according to the Rett Syndrome Severity Scale (RSSS) which consists of seven parameters from 0 (absent/normal) to 3 (severe), and a maximum score of 21 (most severe) [21].

2.4 Molecular analysis

In participants with an identified pathogenic mutation in *MECP2*, no further genetic testing was performed. In participants with identified mutations in other genes than *MECP2*, retesting of

MECP2 with Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) was carried out. Participants with no prior testing were tested with Sanger sequencing and MLPA of *MECP2*. Participants with negative result on earlier analyses were tested with exome-based Next Generation Sequencing (NGS) analysis with bioinformatic filtering of a panel of genes known to cause intellectual disability and/or epileptic encephalopathies. From the spring of 2015 sequence variants were classified according to the ACMG criteria [22]. During the diagnostic period, the number of genes in the diagnostic gene panel for intellectual disability available from the laboratory increased from 57 to above 1400. When the number of genes increased the approach changed from a single patient analysis to a trio analysis, which includes proband, father and mother.

2.5 Statistical analysis

The descriptive analyses included mean and standard deviations or median and inter quartile range for continuous data, and absolute and relative frequencies for categorical data. Continuous data were compared with independent sample t-test and categorical data with chi square test or fisher exact test. Significance level was set to ≤0.05. Statistical analyses were performed using SPSS for Windows version 23.

2.6 Ethics

Ethics approval was obtained from the Regional Committee for Medical Research Ethics, South East Norway (No. 2012/1572). Parental or guardian consent was obtained prior to inclusion.

3. RESULTS

Consent to participate was given on behalf of 93 individuals. Two were excluded due to missing clinical or genetic data, leaving 91 individuals available for analyses. The participants were from 1 to 66 years old, with a median age of 19 (interquartile range 8-30). All geographical parts of Norway were represented, and both rural and urban areas. Half of the participants (53%) lived in the parental home and half (47%) in residential facilities.

3.1 Genetic and clinical investigations

Of the 91 eligible participants 77 had a mutation in *MECP2* and nine had mutations in other genes (Figure 1). Eighty-four individuals fulfilled the diagnostic criteria of RTT. Identified mutations and RTT phenotypic traits as contained in the 2010 diagnostic criteria are presented in Table 1. Four individuals had two mutations in *MECP2* (Supplementary Table 1). The distribution of mutations in *MECP2* is shown in Figure 2. Novel mutations in *MECP2* were reported in 12 individuals and their clinical features are described in Table 2. Global severity

was assessed with the Rett Syndrome Severity Scale, and showed considerable variation (Figure 3).

3.2 Phenotype versus *MECP2* genotype in individuals with RTT

Table 3 shows the characteristics of the individuals with RTT and *MECP2* mutations (n=74) and of the individuals with RTT without an identified *MECP2* mutation (n=10). Classic RTT and loss of both hand skills and language skills were significantly more frequent in individuals with *MECP2* mutations. Grossly abnormal development in the first six months of life was present in six of ten (60.0%) individuals in the non-*MECP2* group, and in three of 74 (4.1%) in the *MECP2* group. Both groups presented with a large number of supplementary criteria, but "eye pointing" was significantly more prevalent in individuals with *MECP2* mutations. In addition, fewer individuals with *MECP2* mutations had early onset of the first seizure and onset of epilepsy before developmental regression.

3.3 Phenotype versus *MECP2* genotype in RTT diagnostic subgroups

Of the 72 individuals with classic RTT 69 (95.8%) had a mutation in *MECP2*. In this subgroup, onset of epilepsy was the only significant difference between the individuals with and without *MECP2* mutations (Table 3). Two of three (66.7%) individuals without an identified mutation in *MECP2* had early onset of epilepsy. In comparison, only one of the 69 (1.4%) individuals with *MECP2* mutations had onset of epilepsy during the first year of life, and three (4.3%) had onset of epilepsy before regression.

Of the twelve individuals with atypical RTT, five (41.7%) had a mutation in *MECP2* (Figure 1). There was a significantly higher prevalence of epilepsy and more often onset of epilepsy before regression in the non-*MECP2* group. Six of seven individuals (85.7%) without *MECP2* mutations presented with epilepsy in the first year of life, compared to one of five individuals (20.0%) with *MECP2* mutations, but this difference did not reach statistical significance (Table 3).

3.4 Phenotype in individuals with RTT with mutations in genes other than *MECP2*

Six of the individuals with RTT had mutations in other genes than *MECP2* (Table 1). Two had a classic RTT phenotype and mutations in *SCN1A*; these are described in a previous publication [23].

A novel and de-novo mutation in *SYNGAP1* was present in one participant. Its pathogenicity was not confirmed, but other missense-mutations in the same triplet are reported as pathogenic [24]. She first presented with seizures at the age of three months, and had daily seizures with multiple seizure types throughout childhood.

One girl had a mutation in *SMC1A*. She had early onset epilepsy with both generalized and focal seizures. During the first years of life she had regular seizures, but from school age her seizures clustered with approximately one week a month with frequent seizures and then some weeks without seizures.

One participant had mutations in *CDKL5*. She experienced her first epileptic seizure at seven weeks of age. After a while she responded well to medications and was seizure-free until 12 months of age. In her seizure-free period, she developed normally but lost many acquired skills and developed hand stereotypies when the seizures returned.

Mutations in *FOXG1* were identified in one participant. Her parents had worried about her development and lack of eye contact from birth. She went through a regression phase at three to four years of age.

4. Discussion

In this cohort with presumed RTT, the use of Next Generation Sequencing to supplement the targeted approach enabled the identification of mutations in six different genes as well as a copy number variant. The genetic heterogeneity in this cohort is in line with other studies [25-27]. The clinical diagnosis of RTT was confirmed in 92% of study participants. The presence of individuals with other conditions in the cohort may be explained by differential diagnostic challenges due to the presence of RTT phenocopies in individuals with intellectual disability or epileptic encephalopathy, and possibly by use of former diagnostic criteria, as many of the individuals had been diagnosed with RTT long before the current diagnostic criteria were published. The finding of a presumed pathogenic mutation in *MECP2* in 88% of individuals with confirmed RTT is in agreement with current knowledge [2]. However, mutations in *MECP2* as well as in *FOXG1* and *CDKL5* were identified both in individuals with confirmed RTT and individuals without, illustrating the impact of the clinical diagnostic criteria.

Comparisons of clinical characteristics in individuals with RTT with and without *MECP2* mutations revealed significant differences in the prevalence of features representing two main inclusion criteria and in one exclusion criterion. In addition, there were significant differences in presence of intense eye gaze and onset of epilepsy. Similar findings have been reported by Charman et al. who found a significantly lower frequency of early onset of both regression and epilepsy in individuals with *MECP2* mutations [18]. Temudo et al. described higher frequency of a regressive period with loss of hand use and language and growth retardation in individuals with *MECP2* mutations, and less intense eye gaze and earlier signs of deviant development and autistic traits in individuals without *MECP2* mutations [17].

The studies of Charman and Temudo did not differentiate between classic and atypical RTT. In classic RTT fulfilling all main and no exclusion criteria are required. Hence, the differences in the features representing these criteria between individuals with and without *MECP2* in the total cohort were not seen in classic RTT. However, such differences were neither found in atypical RTT. The only significant differences between individuals with *MECP2* mutations and others in both subgroups were the lower frequency and a later onset of epilepsy in the individuals with *MECP2* mutations. Two of the three individuals with classic RTT without a *MECP2* mutation had early onset epilepsy, which was almost not seen in classic RTT with *MECP2* mutations. Scientific reports on RTT include descriptions of 18 individuals who fulfill the diagnostic criteria of classic RTT and have mutations in other genes than *MECP2* [5, 9, 16, 27-35]. Onset of epilepsy was described for nine of the 18 individuals, five individuals had an early onset (before one year of age) and six individuals presented with the first seizure before regression [5, 27-29, 31, 32]. This is considerably higher than in the individuals with classic RTT and *MECP2* mutation in the present cohort. Similar results were reported by Nissenkorn and colleagues, none of their participants with early onset of epilepsy had a mutation in *MECP2,* while mutations were found in 87% of those with onset after one year of age [36]. Onset of epilepsy before regression might indicate an influence of epilepsy on the development, like in individuals with developmental and epileptic encephalopathies [37] and contrary to classic RTT with *MECP2* mutations, where seizures seldom precede regression and thus is not likely to contribute to the developmental regression [26].

The three individuals in the present sample with *MECP2* mutation but without RTT apparently had no regression and an overall mild phenotype. For two of these three the absence of a clear regression was the only clinical feature lacking for fulfilling the diagnostic criteria for RTT. With introduction of the 2010 diagnostic criteria, regression became required for diagnosing both classic and atypical RTT [2]. However, this requirement can be questioned for several reasons: in some individuals the regression phase may be so subtle and protracted that it is difficult to register [38], and the regression phase may occur so early in life that it is difficult to observe and recognize. If the early development is deviant, the skills normally lost in regression may not yet have been acquired when the phase of neurophysiological regression occurs [39]. Because regression in the first years of life is a rather inaccurate feature, one may consider revising the criteria and omit developmental regression as a requirement.

Many neurodevelopmental disorders have overlapping phenotypes [5]. Evaluation of the nine individuals in the present cohort with mutations in other genes than *MECP2* revealed that they all had clinical features overlapping with both RTT and other syndromes. Two individuals with a classic RTT phenotype had mutations in *SCN1A,* which are associated with the epileptic encephalopathy of Dravet syndrome. Dravet syndrome is characterized by early onset of severe

epilepsy. In the second year of life, a developmental disorder becomes apparent, and developmental regression may occur [40]. In the present sample, the epilepsy of the two with *SCN1A* mutations was Dravet-like.

One girl with atypical RTT had a mutation in *SMC1A. SMC1A* is one of five genes associated with Cornelia de Lange syndrome, but lately several individuals with *SMC1A* mutations and epileptic encephalopathy have been described, some remarkably RTT-like [5, 33, 41]. The distinct feature of seizure clustering seen in the present girl is also described in other individuals with SMC1A mutations [42].

Another participant with atypical RTT had a mutation in *SYNGAP1*. To our knowledge, an atypical RTT phenotype in individuals with mutations in *SYNGAP1* has not been reported before, although Vidal and associates (2017) point to the similarity between girls with this mutation and Rett syndrome [25]. *SYNGAP1*-associated encephalopathy is categorized as a developmental and epileptic encephalopathy with four main comorbid conditions; intellectual disability, behavioural problems, a high pain threshold and ataxia [24]. In addition, developmental regression is not unusual. The present participant shares these characteristics, except for the behavioural problems [24].

The two individuals with mutations in *CDKL5* shared many clinical characteristics but only one of them had regression, which separated them in terms of diagnosis. However, both participants have several characteristics typical of individuals with the suggested *CDKL5* disorder, such as abnormal early development, early onset of epilepsy and mouthing [43].

Finally, mutations in *FOXG1* were found in two participants. Kortum et al. argues that the early abnormal development, lack of regression and lack of respiratory irregularities in combination with brain imaging features are sufficiently distinct to allow clinical recognition of a FOXG1 syndrome [44]. Both participants had poor eye contact from early infancy, normal breathing patterns and abnormal early development. One showed regression. Unfortunately, the present study did not include MRI scanning.

To sum up, six of the nine individuals with mutations in other genes than *MECP2* fulfilled the diagnostic criteria for RTT. Three individuals did not fulfil the criteria but shared many clinical features with RTT. In addition to RTT, these nine presented with features found in other individuals without RTT but with mutations in the same genes. The current diagnostic criteria for RTT are based on clinical characteristics, a mutation in *MECP2* is neither necessary nor diagnostic, and mutations in other genes do not exclude RTT [2]. Some researchers have suggested replacing the clinical features currently used for diagnosing RTT with a molecular diagnosis [13, 45]. At present, it is not clear what such a change would imply, but it seems evident that it would include a wider phenotypic spectrum than the current criteria. The phenotypes will range from severe RTT to mild intellectual disability, and include the non-Rett

variation among males. Hence, it will lose the benefits a diagnosis based on developmental clinical characteristics features for habilitation and clinical research, as well as for solidarity and support between families. At the same time, the findings from the present study suggest important differences between individuals with and without a mutation in *MECP2.* This may suggest that the current diagnostic criteria include individuals with other disorders under the RTT umbrella.

A limitation in the present article is the relatively small number of participants, the results from this study has to be confirmed by future research involving larger populations. The present sample is however population-based and has a wide distribution in age and geographical location, indicating that it is representative for the population of RTT in Norway, strengthening the external validity in spite of the low number.

In conclusion, both the genotypic and the phenotypic variation within RTT are considerable. The clinical severity are ranging from mild phenotypes with basic language skills, ability to walk and only a few RTT characteristics, to severe phenotypes without ability to speak or to walk independently, and with severe epilepsy. Most individuals had a pathologic mutation in MECP2, but in addition mutations in five other genes were revealed. Compared to individuals with RTT without *MECP2* mutations, individuals with RTT with *MECP2* mutations more often had apparently normal development in the first six months of life, had lost functional use of hands and language, and showed a characteristic intense eye gaze. The prevalence of early onset epilepsy was lower in individuals with a *MECP2* mutation than in individuals without a MECP2 mutation, regardless of which RTT subgroup they belonged to.

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DECLARATIONS OF INTEREST

The authors declare no conflict of interest.

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Table 1. Presence of RTT phenotypic manifestations in individuals with mutations in different genes (number/number in total)

	MECP2	***SCN1A***	***SYNGAP1***	***SMC1A***	***CDKL5***	***FOXG1***	***13q*del**	**No mut. id.**
Number	77	2	1	1	2	2	1	5
Diagnosis								
Classic	69/77	2/2	0/1	0/1	0/2	0/2	0/1	1/5
Atypical	5/77	0/2	1/1	1/1	1/2	1/2	0/1	3/5
Non-RTT	3/77	0/2	0/1	0/1	1/2	1/2	1/1	1/5
Absolute criteria								
Regression	74/77	2/2	1/1	1/1	1/2	1/2	0/1	4/5
Main criteria								
Loss of hand skills	73/77	2/2	0/1	1/1	0/2	1/2	0/1	4/5
Loss of language	73/77	2/2	1/1	0/1	1/2	1/2	uk	2/5
Gait abnormalities	76/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Stereotypies	77/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Exclusion criteria								
Brain injury	0/77	0/2	0/1	0/1	0/2	0/2	0/1	0/5
Grossly abn. developm.	0/77	0/2	1/1	1/1	2/2	2/2	1/1	3/5
Supplementary criteria								
Breathing disturbances	60/76	1/2	1/1	1/1	0/2	0/2	0/1	4/5
Bruxism	60/75	2/2	1/1	1/1	1/2	1/2	1/1	4/5
Impaired sleep	61/77	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Abnormal muscle tone	62/76	2/2	1/1	1/1	2/2	2/2	1/1	5/5
Peripheral vasomotor disturbances	36/73	1/2	1/1	1/1	1/2	2/2	1/1	1/5
Scoliosis/kyphosis	65/77	2/2	0/1	1/1	1/2	2/2	1/1	3/5
Growth retardation	39/75	2/2	0/1	0/1	2/2	2/2	1/1	4/5
Small cold hands/feet	66/75	2/2	1/1	1/1	2/2	2/2	1/1	3/5
Laughter/screaming spells	65/68	2/2	1/1	1/1	2/2	1/2	1/1	5/5
Diminished response to pain	39/43	1/2	1/1	0/1	1/2	2/2	1/1	2/2
Eye pointing	62/63	2/2	1/1	1/1	1/2	1/2	0/1	3/4
Other RTT characteristics								
Microcephaly	37/74	0/2	1/1	1/1	1/2	1/2	1/1	2/5
Verbal language	9/77	0/2	0/1	0/1	0/2	0/2	0/1	0/5
Indep. ambulation	45/77	2/2	1/1	1/1	0/2	0/2	0/1	2/5
Reflux	43/76	1/2	1/1	1/1	1/2	2/2	1/1	3/5
Constipation	70/77	1/2	1/1	1/1	1/2	2/2	1/1	5/5
Epilepsy	50/77	2/2	1/1	1/1	2/2	1/2	1/1	5/5
Onset of epilepsy <1y	2/76	2/2	1/1	1/1	2/2	0/2	1/1	4/5
Onset of epilepsy before regression	4/76	2/2	1/1	1/1	1/2	1/2	na	3/5

No ut. id.: No mutation identified, na: not applicable, uk: unknown

Table 2. RTT phenotypic manifestations of individuals with novel mutations in *MECP2*

	Single nucleotide variation		**Indels**									
Mutation in *MECP2*	**c.872C>T**	**c.1453C>A**	**c.211_1150del**	**c.816_1027del**	**c.817_832dup**	**c.902_1141del**	**c.1064_1196del**	**c.1098_1201del & c.1276_1277dupAG**	**c.1098_1201del & c.1276_1277dupAG**	**c.1127_1197del**	**c.1161_1188del**	**c.1173_1197del**
VUS		Y				Y						
Diagnosis	Non-RTT	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.	Cl.
Absolute criteria												
Regression	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Main criteria												
Loss of hand skills	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Loss of language	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Gait abnormalities	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Stereotypies	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Exclusion criteria												
Brain injury	N	N	N	N	N	N	N	N	N	N	N	N
Grossly abn development	N	N	N	N	N	N	N	N	N	N	N	N
Supplementary criteria												
Breathing disturbances	Y	Y	Y	Y	Y	Y	Y	N	N	Y	Y	Y
Bruxism	Y	N	Y	Y	N	Y	Y	N	N	Y	N	N
Impaired sleep	Y	Y	Y	Y	N	Y	N	Y	N	Y	Y	Y
Abnormal muscle tone	N	N	Y	Y	N	Y	Y	N	N	Y	Y	Y
Periph. vasomotor disturbances	N	Y	Y	Y	N	N	Y	N	N	N	N	Y
Scoliosis/kyphosis	N	N	Y	Y	Y	Y	Y	Y	N	Y	Y	Y
Growth retardation	Y	N	Y	Y	Y	Y	N	N	N	N	N	Y
Small cold hands/feet	Y	Y	Y	Y	Y	N	Y	N	N	Y	Y	Y
Laughter/screaming spells	uk	Y	Y	Y	Y	uk	Y	Y	Y	Y	Y	Y
Diminished response to pain	uk	Y	uk	uk	N	uk	Y	Y	Y	N	uk	Y
«Eye pointing»	Y	Y	uk	Y	Y	Y	Y	Y	Y	Y	Y	Y
Other RTT characteristics												
Microcephaly	Y	N	Y	Y	N	N	Y	N	N	N	N	Y
Verbal language	Y	N	N	N	N	Y	N	Y	Y	N	N	N
Independent ambulation	Y	Y	N	N	N	Y	N	Y	Y	Y	N	N
Reflux	N	Y	N	Y	N	N	N	N	N	Y	Y	Y
Constipation	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y
Epilepsy	N	Y	Y	N	Y	N	N	Y	N	N	Y	Y
Onset of epilepsy (months)	na	6	36	na	144	na	na	60	na	na	108	72
Rett Syndrome Severity score	5	12	17	13	13	10	12	8	6	7	18	13

VUS: variant of unknown significance, Y: yes, N: no, Cl: classic RTT, na: not applicable, uk: unknown

Table 3. Presence of RTT phenotypic manifestations in RTT with and without *MECP2* mutations

	Classic RTT			Atypical RTT			All RTT		
	W. *MECP2*	No *MECP2*	p	W. *MECP2*	No *MECP2*	p	W. *MECP2*	No *MECP2*	p
Number	69	3	-	5	7		74	10	-
Age, mean	23.1	30.0	0.448	21.8	17.3	0.491	23.0	21.1	0.697
Classic RTT	-	-	-	-	-	-	69/74	3/10	<0.001*
Absolute criteria, n/ntotal									
Regression	69/69	3/3	-	5/5	7/7	-	74/74	10/10	-
Main criteria, n/ntotal									
Loss of hand skills	69/69	3/3	-	4/5	4/7	0.576	73/74	7/10	0.005*
Loss of language	69/69	3/3	-	4/5	4/7	0.576	73/74	7/10	0.005*
Gait abnormalities	69/69	3/3	-	4/5	7/7	0.417	73/74	10/10	1.000
Stereotypies	69/69	3/3	-	5/5	7/7	-	74/74	10/10	-
Exclusion criteria, n/ntotal									
Brain injury	0/69	0/3	-	0/5	0/7	-	0/74	0/10	-
Grossly abn. development	0/69	0/3	-	3/5	6/7	0.523	3/74	6/10	<0.001*
Supplementary criteria, n/ntotal									
Breathing disturbances	56/68	1/3	0.097	2/5	5/7	0.558	58/73	6/10	0.226
Bruxism	54/67	3/3	1.000	4/5	4/7	0.576	58/72	7/10	0.425
Impaired sleep	56/69	3/3	1.000	4/5	7/7	0.417	60/74	10/10	0.201
Abnormal muscle tone	56/68	3/3	1.000	5/5	7/7	-	61/73	10/10	0.344
Periph. vasomotor disturbances	33/65	1/3	0.555	2/5	5/7	0.558	35/70	6/10	0.738
Scoliosis/kyphosis	60/69	2/3	0.366	5/5	5/7	0.470	65/74	7/10	0.150
Growth retardation	36/67	3/3	0.599	2/5	5/7	0.558	38/72	4/10	0.514
Small cold hands/feet	58/67	2/3	0.375	5/5	7/7	-	63/72	9/10	1.000
Laughter/screaming spells	59/61	3/3	1.000	5/5	6/7	1.000	64/66	9/10	0.349
Diminished response to pain	35/39	1/2	0.232	3/3	5/6	1.000	38/42	6/8	0.242
"Eye pointing"	54/55	3/3	1.000	5/5	4/6	0.455	59/60	7/9	0.043*
Other RTT characteristics, n/ntotal									
Microcephaly	33/66	0	0.240	3/5	7/7	0.152	36/71	7/10	0.322
Verbal language	4/69	0/3	1.000	2/5	1/7	0.523	6/74	1/10	1.000
Indep. Ambulation	40/69	3/3	0.268	2/5	4/7	1.000	42/74	7/10	0.511
Reflux	39/68	1/3	0.577	4/5	5/7	1.000	43/73	6/10	1.000
Constipation	62/69	2/3	0.301	5/5	6/7	1.000	67/74	8/10	0.290
Epilepsy	48/69	3/3	0.551	2/5	7/7	0.045*	50/74	10/10	0.056
Onset of epilepsy <1y	1/68	2/3	0.004*	1/5	6/7	0.072	2/73	8/10	<0.001*
Onset of epilepsy before regression	3/68	2/3	0.012*	1/5	7/7	0.010*	4/73	9/10	<0.001*
Rett Syndrome Severity Score (mean)	13.2[a]	11.3	0.376	12.8	13.3	0.851	13.2[a]	12.7	0.680

**Significant, a: data from four individuals are missing in this analysis*

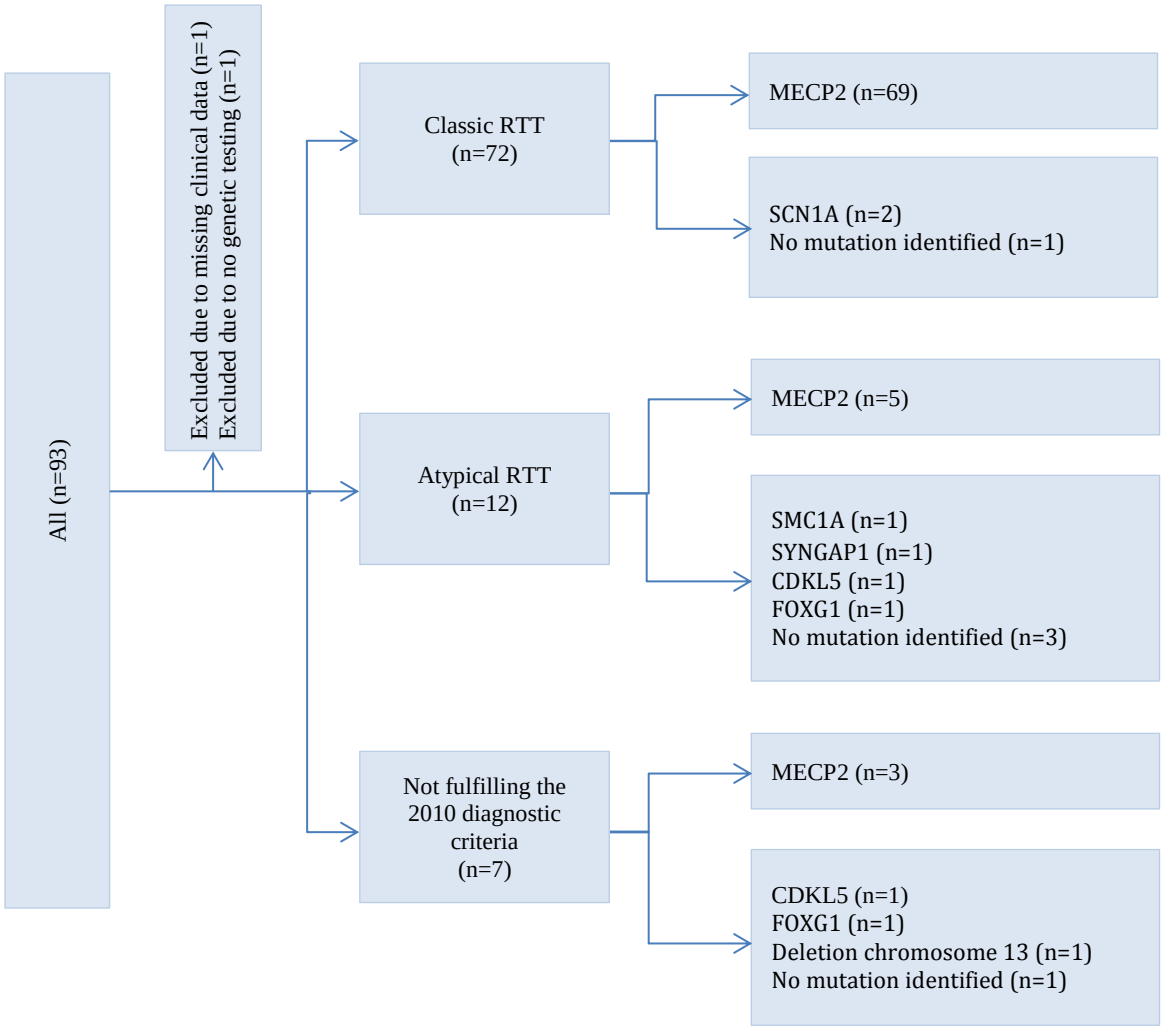
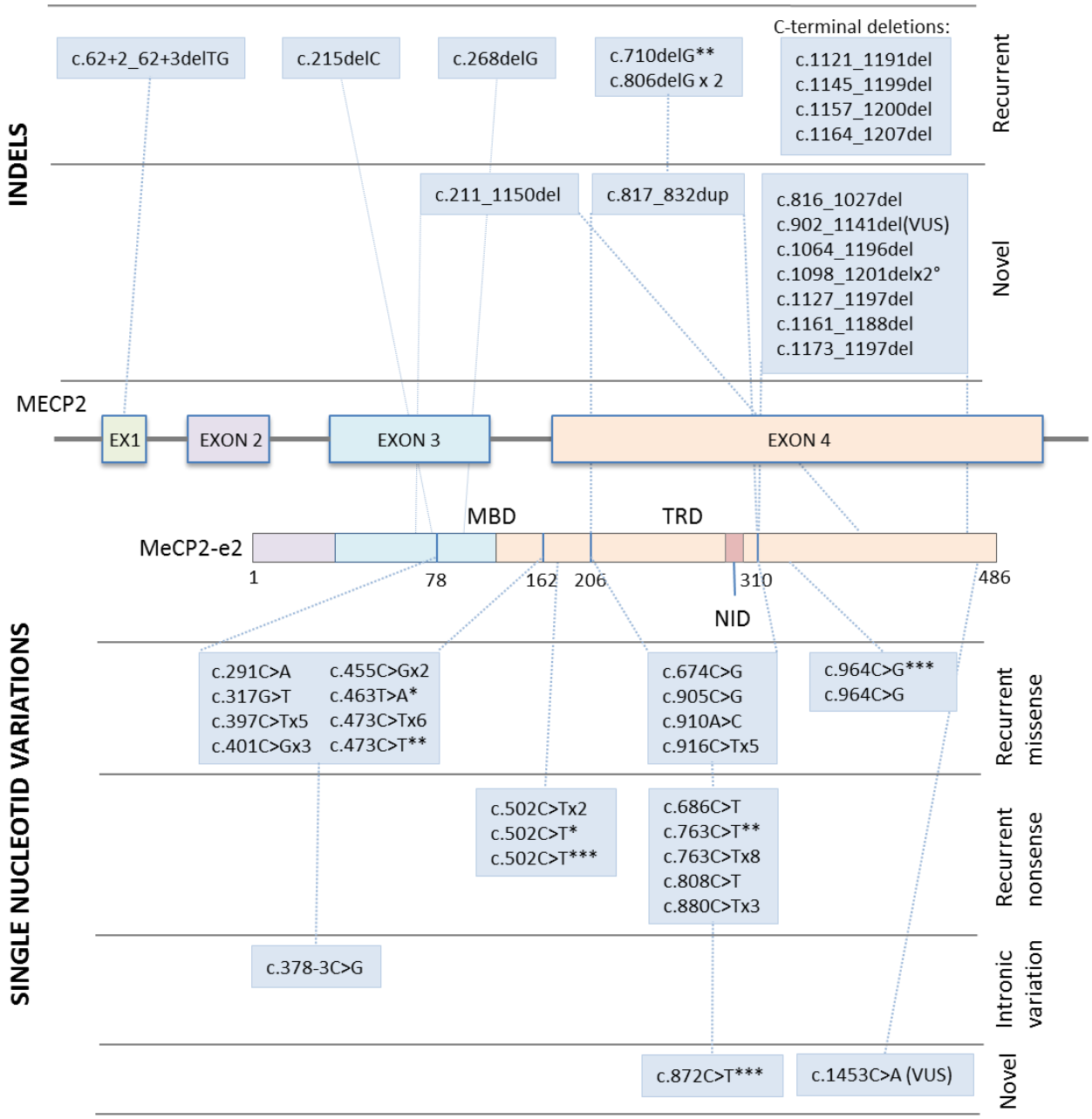


Figure 1. Genotypes and phenotypes in the present sample

a)


INDELS
c.62+2_62+3delTG
c.215delC
c.268delG
c.710delG**
c.806delG x 2
C-terminal deletions:
c.1121_1191del
c.1145_1199del
c.1157_1200del
c.1164_1207del
Recurrent
c.211_1150del
c.817_832dup
c.816_1027del
c.902_1141del(VUS)
c.1064_1196del
c.1098_1201delx2°
c.1127_1197del
c.1161_1188del
c.1173_1197del
Novel
MECP2
EX1
EXON 2
EXON 3
EXON 4
MBD
TRD
MeCP2-e2
1
78
162
206
310
486
NID
SINGLE NUCLEOTID VARIATIONS
c.291C>A
c.317G>T
c.397C>Tx5
c.401C>Gx3
c.455C>Gx2
c.463T>A*
c.473C>Tx6
c.473C>T**
c.674C>G
c.905C>G
c.910A>C
c.916C>Tx5
c.964C>G***
c.964C>G
Recurrent missense
c.502C>Tx2
c.502C>T*
c.502C>T***
c.686C>T
c.763C>T**
c.763C>Tx8
c.808C>T
c.880C>Tx3
Recurrent nonsense
c.378-3C>G
Intronic variation
c.872C>T***
c.1453C>A (VUS)
Novel

b)

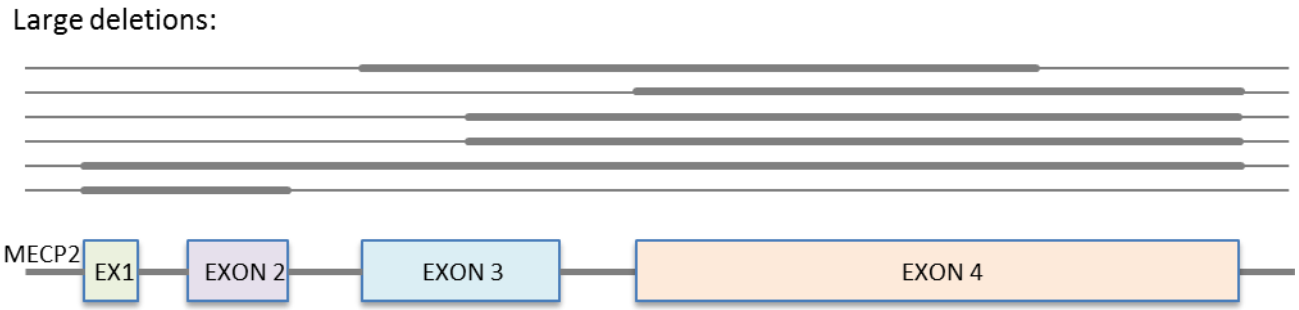


Figure 2. The distribution of mutations in *MECP2* in the present sample illustrated in accordance to the *MECP2* gene and the MeCP2-e2 protein. (The other transcript MeCP2-e1 is for simplicity not included in the figure). In the MeCP2-e2 protein the important functional areas of Methyl-CpG-binding domain (MBD), Transcriptional repression domain (TRD) and NCOR-SMRT interaction domain (NID) are marked, as are the first and last amino acid in MBD and TRD. a) Indels and point mutations of 71 individuals. Their phenotype is marked (**Atypical RTT, mild; **Atypical RTT severe; ***Not fulfilling RTT diagnostic criteria; °Monozygotic twins; All others: classic RTT.*) b) Six individuals had large deletions (illustrated by one line each, the bold lines illustrate the deletion in accordance to the schematic gene). All five had classic RTT.

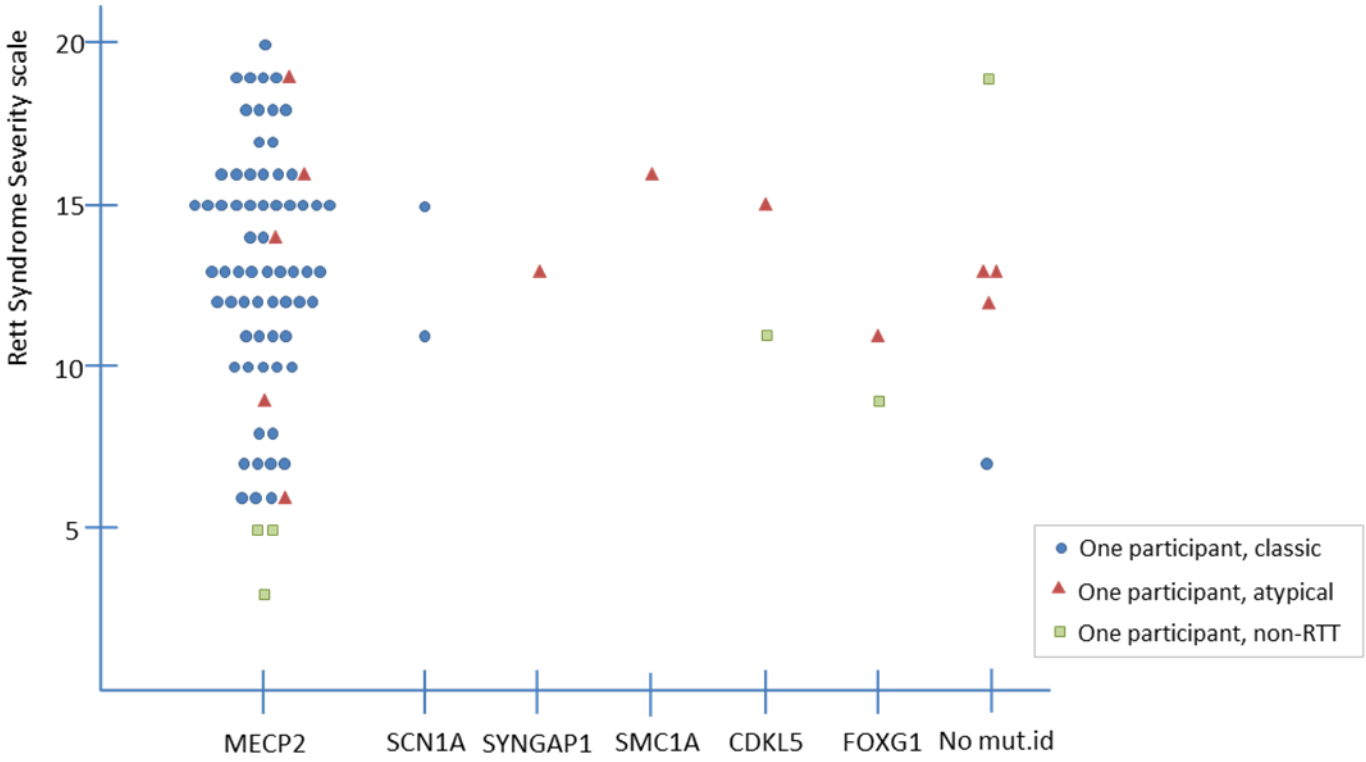


Figure 3. Rett syndrome Severity Scores in individuals divided into groups based on genotype.

Supplementary table 1. Individuals with two mutations in *MECP2*

	Mutation	Novel	Pathogenicity
1	c.910A>C	-	Pathogenic
	c.1123_1191del69	-	Unknown
2*	c.1098_1201del	X	Pathogenic
	c.1276_1277dupAG	X	Likely pathogenic
3*	c.1098_1201del	X	Pathogenic
	c.1276_1277dupAG	X	Likely pathogenic
4	c.964C>G	-	Pathogenic
	c.1145_1199del	X	Likely pathogenic

**monozygotic twins*